# Supplemental Materials

***Molecular Biology of the Cell***

Costa et al.

# Supplemental

## csi2p modulates microtubule dynamics and organizes the bipolar spindle for chromosome segregation.

Judite Costa [1, 2], Chuanhai Fu [3], V. Mohini Khare [1], Phong T. Tran [1, 2]

[1] Department of Cell & Developmental Biology, University of Pennsylvania, Philadelphia PA 19104 USA

[2] Institut Curie, UMR 144 CNRS, Paris 75005 France

[3] Department of Biochemistry, University of Hong Kong, Pokfulam, Hong Kong

Correspondence: tranp@mail.med.upenn.edu

***Content:***

**Figure Legends**

**Figure S1.**

**A.** Colony growth in the presence of the microtubule-depolymerizing drug MBC. csi2Δ cells are more sensitive to MBC than wildtype cells, indicating defects in the microtubule cytoskeleton.

**B.** Time-lapse images of a mitotic csi1Δ cell expressing mCherry-atb2p (tubulin). The csi1Δ cell shows transient microtubule protrusions indicating monopolar spindle phenotype (time=1-9 min), and delayed bipolar spindle formation (time = 10 min). Scale bar, 5μm.

**C.** Box and dot plot comparison of kinesin-5 cut7p spindle recruitment at mitosis onset in wildtype and *csi2Δ* cells. cut7p organizes spindle bipolarity (Hagan and Yanagida, 1992), and shows similar spindle recruitment time for both wildtype and csi2Δ cells, -3.8±1.2 min (n=23) WT versus -3.6±2.2 min (n=16) csi2Δ (p=0.67), suggesting that the csi2Δ phenotype is not due to the improper recruitment of cut7p.

**D.** Box and dot plot comparison of bipolar spindle formation time subsequent to kinesin-5 cut7p recruitment. Wildtype cells establish spindle bipolarity 5.1±1.4 min (n=32) after cut7p recruitment. In contrast, csi2Δ cells take 7.4±2.0 min (n=15) ($p<10^{-3}$).

**Figure S2.**

**A.** Time-lapse images showing centromere segregation in wildtype and csi2Δ cells expressing mCherry-atb2p and CEN1-GFP (centromere marker on chromosome 1). At anaphase B, when the spindle exhibits a dramatic increase in length, the wildtype centromeres are completely segregated to opposite spindle poles. In contrast, there is a lagging centromere (yellow arrow head) in the csi2Δ cell. Bar, 5μm.

**B.** Box and dot plot comparison of the duration of prophase-metaphase for wildtype and csi2Δ cells. Wildtype prophase-metaphase duration is 16.7 ± 3.0min (n=19), similar to 19.1±4.6min (n=20) for csi2Δ (p=0.06).

**C.** Spot assay of cell survival. Single deletion csi2*Δ*, mad2*Δ*, bub3*Δ* and mph1*Δ* grow similarly as wildtype under all temperatures tested. The double-deletion csi2Δmad2Δ, csi2Δbub3Δ and csi2Δmph1Δ exhibit increasing temperature sensitivity and are dead at 37°C, suggesting that the SAC helps ensure proper kinetochore-microtubule attachment in the absence of csi2p.

**Figure S3.**

**A.** Images of wildtype cells expressing csi2-GFP and sid4-mRFP (an SPB marker). csi2p localizes with sid4p at the SPB. Scale bar, 5μm.

**B.** Bar plot comparison of spindle structure in wildtype, csi1Δ, csi2Δ, and double-deletion csi1Δcsi2Δ cells expressing mCherry-atb2p. Compared to csi2Δ, csi1Δ cells showed a much higher frequency of transient monopolar spindles. The double-deletion csi1Δcsi2Δ showed similar frequency of monopolar spindles as csi1Δ, suggesting that csi1p is dominant over csi2p.

**Figure S4.**

**A.** Images of monopolar spindles of cut7.24$^{ts}$ (control) and cut7.24$^{ts}$csi2Δ cells expressing mCherry-atb2p. Bottom panels show enlargements of the spindles from the top panels. Yellow dashed lines outline the spindle and microtubule contour, used to measure spindle area and microtubule intensity. Scale bar, 1μm.

**B.** Box and dot plot comparison of control and csi2Δ monopolar spindle size. Control cells have spindle size of 158±22 sq. pixel (n=24). In contrast, csi2Δ cells have spindle size of 114±39 sq. pixel (n=16), or 28% smaller than control ($p<10^{-3}$).

**C.** Box and dot plot comparison of control and csi2Δ spindle microtubule intensity (arbitrary unit). Control cells have microtubule intensity of 2951±615 a.u. (n=24). In contrast, csi2Δ cells have microtubule intensity of 2009± 526 a.u. (n=16), or 32% less than control ($p<10^{-3}$).

## Table S1. List of Strains

| Strains | Genotype |
|---|---|
| Figure 1 | |
| PT.1939 | mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.2469 | csi2Δ:NatR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.2667 | mCherry-atb2:HygR alp4-GFP:KanR leu1-32 ura4-D18 h? |
| PT.2671 | csi2Δ:NatR mCherry-atb2:HygR alp4-GFP:KanR leu1-32 ura4-D18 h? |
| PT.2973 | cut7-3GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.2975 | csi2Δ:NatR cut7-3GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| | |
| Figure 2 | |
| PT.2482 | cdc13-GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.2483 | csi2Δ:NatR cdc13-GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.2489 | mini chromosome (ch16; ade6-210; his2) ade6-210 leu1-32 ura4-D18 h+ |
| PT.2490 | csi2Δ:NatR mini chromosome (ch16; ade6-210; his2) ade6-210 leu1-32 ura4-D18 h- |
| PT.2513 | mis12-GFP:Leu2 mCherry-atb2:HygR h+ |
| PT.2480 | csi2Δ:NatR mis12-GFP:Leu2 mCherry-atb2:HygR h- |
| | |
| Figure 3 | |
| PT.2505 | csi2-GFP:NatR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.3113 | sad1-YFP:KanR csi2-mCherry:NatR ade6-210 leu1-32 ura4-D18 h? |
| PT.2432 | csi2-mCherry:NatR ade6-210 leu1-32 ura4-D18 h- |
| PT.2673" | csi2-mCherry:NatR sat1.1ts ade6-210 leu1-32 ura4-D18 h? |
| PT.2649 | csi2Δ:NatR sad1-YFP:KanR ade6-210 leu1-32 ura4-D18 h- |
| PT.2688 | csi2-GFP:NatR csi1Δ:KanR mCherry-atb2:HygR ura4-D18 h? |
| CF.671 | csi1-GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.2687 | csi2Δ:NatR csi1-GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h? |
| PT.2513 | mis12-GFP:Leu2 mCherry-atb2:HygR h+ |
| CF.682 | csi1Δ:KanR mis12-GFP:Leu2 mCherry-atb2:HygR ura4-D18 h- |
| PT.2480 | csi2Δ:NatR mis12-GFP:Leu2 mCherry-atb2:HygR h- |
| | |
| Figure 4 | |
| PT.2480 | csi2Δ:NatR mis12-GFP:Leu2 mCherry-atb2:HygR h- |
| PT.3182 | cdc13-GFP:KanR mis6-2mRFP-HygR h- |
| PT.3186 | csi2Δ:NatR cdc13-GFP:KanR mis6-2mRFP-HygR h- |
| | |
| Figure 5 | |
| CF.341 | cut7.24ts mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.3134 | csi2Δ:NatR cut7.24ts mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.3138 | cut7.24ts mis12-GFP:Leu2 mCherry-atb2:HygR h- |
| PT.3141 | csi2Δ:NatR cut7.24ts mis12-GFP:Leu2 mCherry-atb2:HygR h+ |
| | |
| Figure S1 | |
| CF.684 | csi1Δ:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.2973 | cut7-3GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.2975 | csi2Δ:NatR cut7-3GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.286 | wildtype ade6-210 leu1-32 ura4-D18 h- |
| PT.2400 | csi2Δ:NatR ade6-210 leu1-32 ura4-D18 h- |

Figure S2

| | |
|---|---|
| PT.2482 | cdc13-GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.2483 | csi2∆:NatR cdc13-GFP:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| CF.441 | his7+::LacI-GFP lys1+LacO mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.2696 | csi2∆:NatR his7+::LacI-GFP lys1+LacO mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.286 | ade6-210 leu1-32 ura4-D18 h- |
| PT.2400 | csi2∆:NatR ade6-210 leu1-32 ura4-D18 h- |
| PT.3006 | mad2∆:KanR ade6-210 leu1-32 ura4-D18 h+ |
| PT.3005 | bub3∆:KanR ade6-210 leu1-32 ura4-D18 h+ |
| PT.3004 | mph1∆:KanR ade6-210 leu1-32 ura4-D18 h+ |
| PT.2513 | mis12-GFP:Leu2 mCherry-atb2:HygR h+ |
| PT.2481 | csi2∆:NatR mis12-GFP:Leu2 mCherry-atb2:HygR h+ |
| PT.3016 | mad2∆:KanR csi2∆:NatR mis12-GFP:Leu2 mCherry-atb2:HygR h- |
| PT.3014 | bub3∆:KanR csi2∆:NatR mis12-GFP:Leu2 mCherry-atb2:HygR h- |
| PT.3012 | mph1∆:KanR csi2∆:NatR mis12-GFP:Leu2 mCherry-atb2:HygR h- |

Figure S3

| | |
|---|---|
| PT.2646 | csi2-GFP:NatR sid4-mRFP::ura leu1-32 h? |
| CF.684 | csi1Δ:KanR mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.1939 | mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.2469 | csi2Δ:NatR mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.2684 | csi1Δ:KanR csi2Δ:NatR mCherry-atb2:HygR leu1-32 ura4-D18 h? |

Figure S4

| | |
|---|---|
| CF.341 | cut7.24ts mCherry-atb2:HygR leu1-32 ura4-D18 h+ |
| PT.3134 | csi2Δ:NatR cut7.24ts mCherry-atb2:HygR leu1-32 ura4-D18 h- |
| PT.3138 | cut7.24ts mis12-GFP:Leu2 mCherry-atb2:HygR h- |
| PT.3141 | csi2Δ:NatR cut7.24ts mis12-GFP:Leu2 mCherry-atb2:HygR h+ |

Figure S1, Costa et al

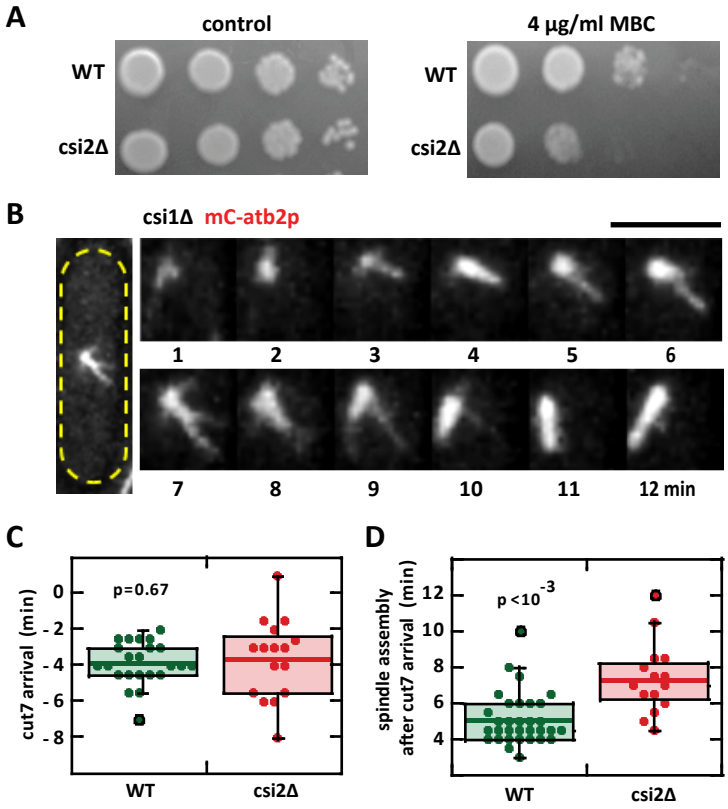

Figure S2, Costa et al

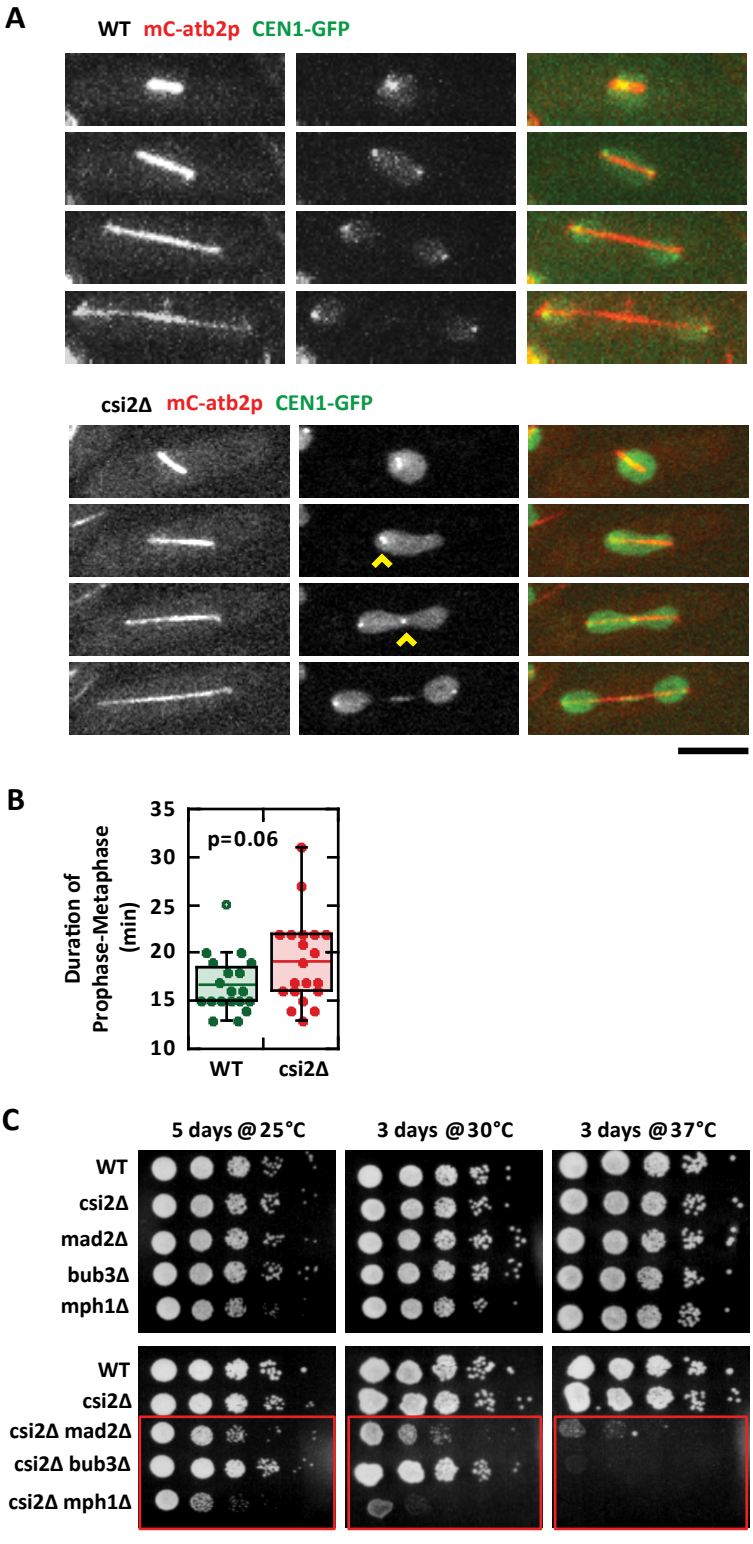

**Figure S3, Costa et al**

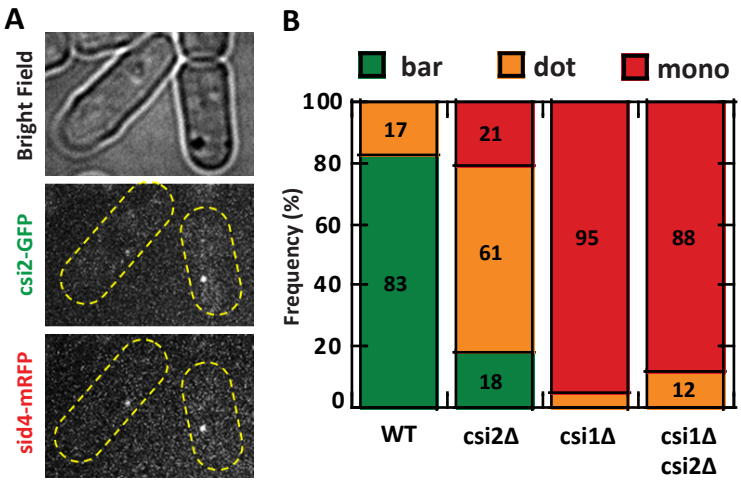

**Figure S4, Costa et al**